

IN VITRO AND IN VIVO EVALUATION OF ETHNOVETERINARY HERBAL PREPARATIONS FOR ANTHELMINTIC ACTIVITY AGAINST GASTROINTESTINAL NEMATODES IN SHEEP

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ABSTRACT

Gastrointestinal nematodes are an important constraint to small-ruminant production, and the increasing concern about anthelmintic resistance has encouraged investigation of locally available ethnoveterinary preparations. This study documented traditional deworming practices in Tirunelveli District, Tamil Nadu, and evaluated selected herbal preparations in vitro and in sheep. Local healers, farmers, women's self-help groups and community representatives were consulted to document plant species, plant parts and preparation methods. Aqueous and ethanolic extracts were assessed using an egg-hatch assay at 10, 20, 40 and 80 mg/mL. In the in vitro test, aqueous Aloe vera extract showed reported inhibition values of 88.50, 92.75, 94.00 and 97.75% at 10, 20, 40 and 80 mg/mL, respectively. At 80 mg/mL, the combined herbal preparation with A. vera showed 94.00% inhibition in the aqueous extract and 94.75% in the ethanolic extract; the reported positive-control value was 95.50%. In the in vivo faecal egg count reduction test, the day-12 reductions were $74.96 \pm 0.81\%$ for A. vera, $92.58 \pm 1.22\%$ for the herbal mixture, $94.04 \pm 0.81\%$ for the combined preparation and $94.09 \pm 0.53\%$ for fenbendazole. No abnormal clinical signs were reported during the 24-hour observation period after treatment. These findings indicate that the combined ethnoveterinary preparation merits further investigation as a potential anthelmintic option. However, the results require confirmation using a clearly specified experimental design, validated assay terminology, appropriate safety evaluation and larger controlled field trials.

KEYWORDS: Aloe vera; anthelmintic activity; ethnoveterinary medicine; faecal egg count reduction; gastrointestinal nematodes; sheep

INTRODUCTION

Gastrointestinal nematode infections are a major health and production concern in sheep and goats, particularly in tropical and subtropical production systems. Infection can reduce growth, body condition and productivity and, in severe cases, contribute to morbidity and mortality. Control has traditionally relied on synthetic anthelmintics, but the development and spread of resistance have reduced the reliability of some treatments and increased the need for sustainable parasite-management strategies (Geerts and Gryseels, 2000; Sangster, 1999; Coles et al., 2006).

Ethnoveterinary medicine represents a body of locally developed knowledge used by livestock keepers to manage animal health. Plant-based preparations remain accessible in some rural communities because their ingredients may be locally available and familiar to farmers. Surveys in different parts of India, including Tamil Nadu, have documented the use of medicinal plants for livestock ailments, although traditional use alone does not establish efficacy or safety (Ganesan et al., 2008; Reddy et al., 2006; Athanasiadou et al., 2007).

Plant extracts may contain several classes of biologically active compounds, including tannins, saponins, flavonoids and other secondary metabolites. Their effects on parasites can vary with plant species, plant part, extraction solvent, concentration and parasite stage. Laboratory screening can identify preparations warranting further investigation, while controlled animal studies are needed to determine whether laboratory activity translates into meaningful reductions in parasite burden (Hammond et al., 1997; Egualde et al., 2006; Kamaraj and Rahuman, 2011).

The original project documented deworming practices used by local healers in Tirunelveli District and compared selected preparations with fenbendazole in sheep. The plants frequently mentioned during the survey included *Azadirachta indica*, *Andrographis paniculata*, *Aloe vera* and *Sesbania grandiflora*. The present

manuscript summarizes the documented survey, in vitro screening and in vivo faecal egg count findings, with particular attention to Aloe vera and a combined herbal preparation. The objectives were to document locally used ethnoveterinary deworming preparations, assess selected extracts in vitro, and compare faecal egg count reduction following treatment in naturally infected sheep.

MATERIALS AND METHODS

Study area and ethnoveterinary survey

The study was conducted as part of a Tamil Nadu Veterinary and Animal Sciences University Research Corpus Fund project during 2014–2015. The survey was undertaken in selected villages of Tirunelveli District, Tamil Nadu. Discussions were held with local healers, farmers, women's self-help groups, community representatives and other local stakeholders. Veterinary personnel and representatives of a non-governmental organization were consulted during planning to explain the purpose of the survey and identify relevant communities. Information was collected on plants used for deworming, local names, plant parts, preparation methods and modes of administration.

Plant material and preparation of herbal formulations

Thirteen plant materials were reported as selected for investigation. Plant materials were collected, identified, shade-dried and powdered according to the project report. Aloe vera was evaluated separately as well as in combination with a multi-ingredient herbal preparation. The report describes the combined preparation as containing cumin, mustard, turmeric, pepper, garlic, fenugreek and ginger at 50 g each, together with Thumbai leaves, neem leaves, banana stem, bitter gourd and Nila vembu at 100 g each. Jaggery was added at 100 g per 10 kg body weight for administration, as described in the source report. Botanical identification and voucher specimen details should be added if available.

Preparation of aqueous and ethanolic extracts

Aqueous and ethanolic extracts were prepared from the selected herbal materials for in vitro evaluation. The reported extraction yields were 19.82% for the aqueous extract and 1.120% for the ethanolic extract. The original report available for manuscript preparation does not provide enough unambiguous detail to reproduce the extraction procedure fully. Before submission, the author should insert the exact plant-to-solvent ratio, extraction duration, temperature, filtration procedure, concentration method, storage conditions and the basis used to calculate extraction yield from laboratory records.

Qualitative phytochemical screening

Qualitative screening was reported for flavonoids, alkaloids, terpenoids, sterols, carotenoids, tannins, saponins, reducing sugars, anthraquinones, glycosides, proteins and phenolic compounds using the procedures cited in the original project report. The reported screening indicated that alkaloids were absent from the aqueous extracts but present in the ethanolic extracts. The detailed test-specific results and the precise protocols should be checked against the original laboratory records before publication.

In vitro egg-hatch assay

The project report describes an egg-hatch assay using parasite eggs isolated from faeces. Faecal material was passed through sieves of 500, 75 and 35 μm , followed by centrifugation and flotation using saturated sodium chloride; recovered eggs were washed before use. Extracts were evaluated at 10, 20, 40 and 80 mg/mL in 24-well plates, with approximately 100 eggs in 100 μL per well. Fenbendazole at 0.1 $\mu\text{g/mL}$ was used as the positive control and distilled water as the negative control. Plates were incubated at 26°C for 48 h. The report cites Jackson et al. (2001), but that reference describes a larval migration inhibition assay rather than an egg-hatch assay. The assay protocol and citation must therefore be verified and corrected before submission.

The report presents the endpoint as percentage egg-hatch inhibition. It also describes calculating hatch as larvae divided by the sum of larvae and eggs, multiplied by 100. These are not identical endpoints; the original worksheets must be checked to establish whether the tabulated values represent percentage hatch or percentage inhibition of hatch. The final method and formula should accurately reflect the calculation actually performed.

In vivo faecal egg count reduction test

Naturally infected sheep with baseline faecal egg counts reported in the range of 1,000–1,700 eggs per gram (EPG) were assigned to five groups: T1, untreated infected control; T2, fenbendazole at 7.5 mg/kg body weight; T3, Aloe vera at 100 g/10 kg body weight; T4, the multi-ingredient herbal preparation; and T5, the multi-ingredient preparation combined with Aloe vera. Faecal samples were collected before treatment (day 0) and on day 12 after treatment. Faecal egg counts were determined using a modified McMaster technique, with Coles et al. (1992) cited in the report.

Faecal egg count reduction was calculated as: $\text{FECR (\%)} = [(\text{mean pre-treatment EPG} - \text{mean post-treatment EPG}) / \text{mean pre-treatment EPG}] \times 100$. The report states that sheep were observed for 24 h after treatment for abnormal clinical signs, including allergy, salivation and enteritis, and that grazing and watering behaviour was monitored. The exact number of animals per group is ambiguous in the report and must be confirmed from the original records before submission. The exact administration route and frequency should also be inserted if recorded.

Statistical analysis

The project report states that data were analysed by one-way analysis of variance followed by Duncan's multiple range test using SPSS version 17.0, with $P < 0.05$ considered statistically significant. The statistical

unit, sample size per group, treatment of repeated measurements and whether the reported variability represents standard error or standard deviation should be confirmed before final submission.

RESULTS

Ethnoveterinary survey

The survey documented traditional plant-based preparations used by local healers and livestock keepers for deworming small ruminants in Tirunelveli District. The plant parts reported for medicinal use included leaves, bark, rhizomes, roots, seeds, stems and fruits. Common preparation forms included pastes, powders, decoctions and mixtures of multiple plant materials. The project report identified *Azadirachta indica* (neem), *Andrographis paniculata* (including locally used names associated with Siriyangai/Nila vembu), *Aloe vera* (Katrallai) and *Sesbania grandiflora* (Agathi) among the plants commonly mentioned. The report also noted that only a limited number of stakeholders retained knowledge of these practices.

Extraction yield and phytochemical screening

The reported extraction yields were 19.82% for aqueous extraction and 1.120% for ethanolic extraction. Qualitative screening covered multiple phytochemical classes. According to the report, alkaloids were not detected in aqueous extracts but were detected in ethanolic extracts. The complete plant-by-plant phytochemical results should be inserted from the original data sheets if the manuscript is to report these findings in detail.

In vitro activity

The report's tabulated values showed concentration-related increases in the reported inhibition endpoint for *Aloe vera* aqueous extract, from $88.50 \pm 1.04\%$ at 10 mg/mL to $97.75 \pm 0.63\%$ at 80 mg/mL. The corresponding ethanolic extract values were lower, increasing from $13.75 \pm 1.11\%$ to $23.75 \pm 1.38\%$. The multi-ingredient herbal mixture showed reported values of $55.00 \pm 2.48\%$ to $78.75 \pm 2.02\%$ for the aqueous extract and $50.75 \pm 3.75\%$ to $76.75 \pm 1.25\%$ for the ethanolic extract across the same concentration range. The combined herbal mixture and *Aloe vera* preparation showed $94.00 \pm 2.48\%$ in the aqueous extract and $94.75 \pm 0.85\%$ in the ethanolic extract at 80 mg/mL. The positive-control value was reported as $95.50 \pm 0.96\%$. These values are reproduced from the project report; the endpoint terminology requires verification as noted in Materials and Methods.

Preparation	Concentration (mg/mL)	Aqueous extract (%)	Ethanolic extract (%)
<i>Aloe vera</i>	10	88.50 ± 1.04	13.75 ± 1.11
<i>Aloe vera</i>	20	92.75 ± 0.48	17.25 ± 0.75
<i>Aloe vera</i>	40	94.00 ± 0.91	19.00 ± 1.47
<i>Aloe vera</i>	80	97.75 ± 0.63	23.75 ± 1.38
Herbal mixture	10	55.00 ± 2.48	50.75 ± 3.75
Herbal mixture	20	69.25 ± 3.73	66.00 ± 0.91
Herbal mixture	40	74.25 ± 2.78	69.25 ± 1.03
Herbal mixture	80	78.75 ± 2.02	76.75 ± 1.25
Herbal mixture + <i>Aloe vera</i>	10	33.75 ± 1.55	48.25 ± 2.56
Herbal mixture + <i>Aloe vera</i>	20	82.00 ± 1.96	72.25 ± 0.75
Herbal mixture + <i>Aloe vera</i>	40	90.50 ± 0.65	85.00 ± 1.96
Herbal mixture + <i>Aloe vera</i>	80	94.00 ± 2.48	94.75 ± 0.85
Positive control	As reported	95.50 ± 0.96	95.50 ± 0.96

Table 1. Reported in vitro values for the egg-hatch assay endpoint. The original report's endpoint label and calculation should be reconciled before publication.

In vivo faecal egg count reduction

At day 12 after treatment, the reported faecal egg count reduction was $94.09 \pm 0.53\%$ for fenbendazole, $74.96 \pm 0.81\%$ for *Aloe vera*, $92.58 \pm 1.22\%$ for the herbal mixture and $94.04 \pm 0.81\%$ for the combined herbal mixture plus *Aloe vera*. The untreated control showed an increase in mean EPG from $1,327.75 \pm 25.12$ before treatment to $1,382.40 \pm 27.86$ after the observation period. The report indicates that the reductions for fenbendazole, the herbal mixture and the combined preparation were not significantly different from one another, whereas *Aloe vera* alone produced a significantly lower reduction. These statistical interpretations should be checked against the original analysis output and sample sizes.

Group / treatment	Pre-treatment EPG	Day-12 EPG	Reduction (%)	Reported significance
T1: Untreated control	1327.75 ± 25.12	1382.40 ± 27.86	Not reported	—
T2: Fenbendazole	1377.50 ± 38.35	82.20 ± 7.72	94.09 ± 0.53	a
T3: <i>Aloe vera</i>	1415.40 ± 34.69	353.45 ± 13.21	74.96 ± 0.81	b
T4: Herbal mixture	1349.00 ± 39.55	99.50 ± 15.60	92.58 ± 1.22	a

T5: Herbal mixture + Aloe vera	1451.15 ± 29.49	88.05 ± 12.36	94.04 ± 0.81	a
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Table 2. Reported faecal egg counts and day-12 reduction in naturally infected sheep. EPG, eggs per gram of faeces. Values are reproduced from the source report. Confirm whether variability is standard error or standard deviation and verify the number of animals per group. Different superscript letters were reported to indicate a significant difference ($P < 0.05$).

Clinical observations

The project report recorded no abnormal clinical signs suggestive of allergy, excessive salivation or enteritis during the 24 h observation period after treatment. Treated sheep were reported to maintain normal grazing and watering behaviour. These short-term observations do not establish comprehensive or long-term safety.

DISCUSSION

Ethnoveterinary knowledge and local practices

The survey documented a range of locally used plant materials and preparation methods for deworming small ruminants. The use of leaves, bark, roots, seeds, stems and fruits, and the preparation of pastes, powders, decoctions and mixtures, illustrates the diversity of ethnoveterinary approaches in the study area. Similar documentation studies from India and elsewhere have emphasized the value of recording community knowledge while evaluating individual remedies through reproducible scientific methods (Ganesan et al., 2008; Reddy et al., 2006; Hammond et al., 1997). Traditional use is a starting point for investigation, rather than proof of efficacy.

Extraction and phytochemical findings

The higher reported yield from aqueous extraction than ethanolic extraction indicates that the solvent system affected the quantity of extract recovered. The reported difference in alkaloid detection between aqueous and ethanolic extracts further suggests that extraction solvent may influence the phytochemical profile. However, qualitative phytochemical tests are preliminary and do not identify individual active constituents or establish their concentrations. Future studies should use validated analytical methods, quantify candidate compounds and standardize the preparation to reduce variation between batches.

In vitro anthelmintic findings

The project report recorded high activity for aqueous Aloe vera extract at the highest tested concentration and substantial activity for the combined herbal preparation in both extraction systems. The herbal mixture alone showed a more moderate response. The apparent concentration-related pattern supports additional laboratory investigation, but the endpoint must first be resolved: percentage egg hatch and percentage inhibition of egg hatch are different measures and cannot be used interchangeably. The cited Jackson et al. (2001) method concerns larval migration inhibition, so it should not be retained as the sole method citation for an egg-hatch assay without checking the original protocol. Egg-hatch assays have been used to investigate nematode responses to anthelmintics, including the method described by Le Jambre (1976), but the final citation must match the exact procedure performed.

In vivo efficacy

The combined preparation produced a reported day-12 faecal egg count reduction of $94.04 \pm 0.81\%$, close to the $94.09 \pm 0.53\%$ reported for fenbendazole. The herbal mixture alone also showed a high reported reduction, whereas Aloe vera alone was less effective. These findings are promising as an initial evaluation of the preparations used in the study area. Nevertheless, numerical similarity in percentage reduction does not demonstrate equivalence or non-inferiority to fenbendazole. Such a conclusion would require a prospectively defined design, adequate sample size, appropriate statistical analysis and confidence intervals.

The untreated group's mean EPG increased over the observation period, which is consistent with ongoing infection in the absence of treatment. Interpretation of treatment effects remains dependent on confirmation of group size, randomization, baseline comparability, the statistical unit and the exact method used to calculate reductions. The parasite genera and species present were described in the original report's background, but species-level attribution of the measured response should not be made unless supported by coproculture, larval identification or another documented diagnostic method.

Safety and limitations

The absence of overt clinical signs during the first 24 h after administration is a limited observation and should not be interpreted as proof of safety. The report does not provide sufficient detail to establish comprehensive toxicological evaluation, longer-term follow-up, clinical chemistry, haematology or residue-related considerations. In addition, the exact number of sheep per treatment group and some formulation and extraction details are ambiguous. The study was conducted as a project in 2014–2015, and its findings should be interpreted as historical experimental observations requiring independent replication.

Future research

Further work should begin with confirmation of the original raw data and assay calculations, followed by standardized formulation and phytochemical characterization. *In vitro* testing should include suitable positive and negative controls, documented replicates and a validated assay protocol. Controlled animal trials should use a justified sample size, random allocation, predefined outcomes, appropriate ethics approval, longer safety monitoring and a recognised faecal egg count reduction analysis. Field studies across farms and seasons would be needed to evaluate reproducibility, practical administration, farmer acceptability and cost-effectiveness.

CONCLUSION

The original project documented ethnoveterinary deworming practices in Tirunelveli District and reported anthelmintic activity for Aloe vera and a combined herbal preparation. The combined preparation showed a day-12 faecal egg count reduction similar in magnitude to the fenbendazole value reported in the study, while Aloe vera alone showed a lower reduction. These findings justify further investigation but do not establish therapeutic equivalence or comprehensive safety. Confirmation of the original methods and data, standardized formulation, appropriate ethics documentation and well-designed controlled trials are required before recommending the preparations for routine use.

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